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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/380,447	09/01/1999	Sachdev S. Sidhu	P1581R2	2633

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EXAMINER	
TRAN, MY CHAU T	

ART UNIT	PAPER NUMBER
1639	

DATE MAILED: 03/09/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/380,447	SIDHU ET AL.	
	Examiner	Art Unit	
	MY-CHAU T. TRAN	1639	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *18 November 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,3,4,7-9,11,12,29-33,44-49 and 52-58* is/are pending in the application.

4a) Of the above claim(s) *29,48,49 and 52-54* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3,4,7-9,11,12,30-33,44-47 and 55-58* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *01 September 1999* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *11/18/05 & 2/26/04*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 11/18/2005 has been entered.

Application and Claims Status

2. Applicant's amendment and response filed 11/18/2005 is acknowledged and entered. Claims 1 and 31 have been amended. Claims 55-58 have been added.

3. The amendment filed on 08/22/2005: cancelled claim 42 and amended the specification to note the correct priority of this application.

4. The amendment filed on 6/14/2004: cancelled claims 34-41, 43, 50, and 51; amended claims 31; and added claims 52-54.

5. The amendment filed on 2/26/2004: cancelled claims 2, 5, 6, 10, and 13-28; amended claims 1, 11, and 12; and added claims 29-51.

6. Claims 1, 3, 4, 7-9, 11, 12, 29-33, 44-49, and 52-58 are pending.

Election/Restrictions

7. The instant species election requirement is still in effect as there is no allowable generic or linking claim. Applicant has elected the following species for the elected invention (Claims 1, 3, 4, 7-9, 11, 12, 29-33, 44-49, and 52-58) in the reply filed on 11/02/2004:

a. For the single specific species of a major coat protein, applicant elected a filamentous phage of gp VIII, i.e. wild type M13 with the sequence of SEQ ID NO. 2.

b. For the single specific species of variant of the major coat protein, applicant elected variant of the major coat protein, i.e. wild type M13 with the sequence of SEQ ID NO. 2, wherein the amino acid and its position are as follows: Position No./Amino Acid: 1/D, 2/K, 3/S, 4/E, 5/K, 6/F, 7/S, 8/R, 9/D, 11/Y, 12/E, 13/A, 14/L, 15/E, 16/D, 17/I, 18/I, 19/T, 20/N, 21/L, 22/F, 23/F, 24/L, 25/L, 26/G, 27/T, 28/V, 29/Y, 30/V.

c. For the single specific species of heterologous protein, applicant elected an antibody or fragment thereof.

d. For the single specific species of linking peptide, applicant elected SEQ ID NO. 110.

e. For the single specific species of target, applicant elected erb 2.

8. Applicant's election of species in the reply filed on 11/2/2004 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

9. Claims 29, 48, 49, and 52-54 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to ***non-elected species***, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 11/2/2004.

10. Claims 1, 3, 4, 7-9, 11, 12, 30-33, 44-47, and 55-58 are under consideration in this Office Action.

Priority

11. This instant application is a 371 of PCT/US99/16,596 filed 7/22/1999, which claims benefit to four provisional applications. They are 60/094,291 filed 07/27/1998, 60/103,514 filed 10/08/1998, 60/133,296 filed 05/10/1999, and 60/134,870 filed 05/19/1999. However, the instant claims 1, 3, 4, 7-9, 11, 12, 30-33, 44-47, and 55-58 are granted the benefit of priority for 60/103,514 filed 10/08/1998 and 60/134,870 filed 05/19/1999 under 35 U.S.C. 119(e).

12. It is noted in the response filed 08/22/2005 that applicant also requested for a corrected filling receipt with regard to the claimed benefit for the four provisional applications. The application will be forwarded to the Office of Initial Patent Examination (OIPE) for issuance of a corrected filing receipt, and correction of Office records to reflect the claimed benefit for the four provisional applications as corrected.

Information Disclosure Statement

13. The information disclosure statement (IDS) filed on 11/18/2005 has been reviewed, and its references have been considered as noted on PTO-1449 form(s). In addition, the references of US Patents 6,054,312 and 6,190,908 B1 were consider by the examiner for the IDS filed 02/26/2004 and it is noted that the PTO-1449 form mailed to the applicant was not properly signed and dated by the examiner. The properly signed and dated PTO-1449 form for the IDS filed 02/26/2004 is enclosed, and the examiner apologizes for the inconvenience.

14. Please note: Applicant's *specifically* elected species of a fusion protein, which comprises a variant of filamentous phage of gp VIII, i.e. wild type M13 with the sequence of SEQ ID NO. 2 wherein its amino acid variant at the following position are as follows: (Position No./Amino Acid) 1/D, 2/K, 3/S, 4/E, 5/K, 6/F, 7/S, 8/R, 9/D, 11/Y, 12/E, 13/A, 14/L, 15/E, 16/D, 17/I, 18/I, 19/T, 20/N, 21/L, 22/F, 23/F, 24/L, 25/L, 26/G, 27/T, 28/V, 29/Y, 30/V; a linking peptide of SEQ ID NO. 110; and an antibody that binds to erb 2 (see paragraph 6 above). The elected species of a fusion protein was searched and was not found in the prior art. Also, see MPEP § 803.02 (emphasis added):

> On the other hand, should no prior art be found that anticipates or renders obvious the elected species, the search of the Markush-type claim will be extended. If prior art is then found that anticipates or renders obvious the Markush-type claim with respect to a nonelected species, the Markush-type claim shall be rejected and claims to the nonelected species held withdrawn from further consideration. ***The prior art search, however, will not be extended unnecessarily to cover all nonelected species.*** Should applicant, in response to this rejection of the Markush-type claim, overcome the rejection, as by amending the Markush-type claim to exclude the species anticipated or rendered obvious by the prior art, the amended Markush-type claim will be reexamined. The prior art search will be extended to the extent necessary to determine patentability of the Markush-type claim. In the event prior art is found during the reexamination that anticipates or renders obvious the amended Markush-type claim, the claim will be rejected and the action >can be< made final >unless the examiner introduces a new ground of rejection that is neither necessitated by applicant's amendment of the claims nor based on information submitted in an information disclosure statement filed during the period set forth in 37 CFR 1.97(c) with the fee set forth in 37 CFR 1.17(p). See MPEP § 706.07(a)<. Amendments submitted after the final rejection further restricting the scope of the

> claim may be denied entry >if they do not comply with the requirements of 37 CFR 1.116. See MPEP § 714.13.

Thus the search was expanded to non-elected species, which *were* found in the prior art; see rejections below.

Claim Rejections - 35 USC § 112

15. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

16. Claims 1, 3, 4, 7-9, 11, 12, 30-33, 44-47, and 55-58 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claims contains subject matter, which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a written description rejection.

The instant claim 1 recites a fusion protein, i.e. a product. Structurally, the product comprises a heterologous polypeptide fused to at least a portion of a variant of a wild type major coat protein of a virus. The variant of a wild type major coat protein of a virus is selected from the group consisting of a filamentous phage, a lambda phage, a Baculovirus, a T4 phage and a T7 phage.

As claimed, the variant is a broad genus wherein there is no core structure, i.e. there is no identification of any particular portion of the structure that must be conserved for the instant

claimed variant. Although the claimed variant also claimed that it is from the wild type major coat protein of a virus, i.e. phage display systems, that include the phage display systems of a filamentous phage, a lambda phage, a Baculovirus, a T4 phage and a T7 phage, this limitation is not sufficient to provide a core structure since there is a substantial variation within the genus of these 'different' phage display systems (i.e. a filamentous phage, a lambda phage, a Baculovirus, a T4 phage and a T7 phage) such that the structure of the wild type major coat proteins among these 'different' phage display systems are structurally distinct from each other, i.e. there is no structural nexus among these wild type major coat proteins of these 'different' phage display systems. Therefore, the claimed variant is a broad genus wherein there is no core structure.

The instant specification disclosure does not sufficiently teach the broad genus for the claimed variant of wild type major coat protein of phage display systems that include the phage display systems of a lambda phage, a Baculovirus, a T4 phage and a T7 phage. The instant specification disclosure provides the definition of the terms "variant" and "mutant" (see pg. 19, lines 16-35), the listing of phage display systems such as filamentous phage, lambda phage, Baculovirus, T4 phage and T7 phage (see pg. 36, lines 22-37; pg. 43, lines 13-14), and the statement that the wild type major coat protein of these phage display systems (i.e. a lambda phage, a Baculovirus, a T4 phage and a T7 phage) can be mutated to form the variant of a wild type major coat protein (see pg. 43, lines 14-19). The instant specification description is directed to the variant of wild type major coat protein of the filamentous phage, i.e. the major coat protein VIII of a filamentous phage (see pg. 39, line 1 thru pg. 43, line 12). The instant sequence listing discloses the wild type major coat protein of the filamentous phage, i.e. SEQ ID NO. 2, 3, 4, 5, 6, 7, and 8, which are also claimed in claim 31. The specification examples are drawn to the

method of making the variant of wild type major coat protein VIII of the filamentous phage (see pgs. 72-74, Example 10-12; pgs. 80-83, Examples 24-25) and the method of using the variant of wild type major coat protein VIII of the filamentous phage (see pgs. 74-77, Example 13-21; pgs. 84-85, Example 26). These disclosure clearly does not provide an adequate representation regarding the broad genus for the claimed variant of wild type major coat protein of phage display systems that include the phage display systems of a lambda phage, a Baculovirus, a T4 phage and a T7 phage because there is a substantial variation within the genus of these 'different' phage display systems, i.e. a filamentous phage, a lambda phage, a Baculovirus, a T4 phage and a T7 phage, such that the structure of the wild type major coat proteins among these 'different' phage display systems are structurally distinct from each other, i.e. there is no structural nexus among there wild type major coat proteins of these 'different' phage display systems. Consequently, the instant specification disclosure does not sufficiently teach the broad genus for the claimed variant of wild type major coat protein of phage display systems that include the phage display systems of a lambda phage, a Baculovirus, a T4 phage and a T7 phage.

To provide adequate written description and evidence of possession of a claimed genus, the specification must provide sufficient distinguishing identifying characteristics of the genus. The factors to be considered include disclosure of complete or partial structure, physical and/or chemical properties, functional characteristics, structure/function correlation, methods of making the claimed product, or any combination thereof. In this case, the only factor present for the claimed variant is their functions for binding and displaying a heterologous polypeptide. There is not even identification of any particular portion of the structure that must be conserved among the claimed variant, i.e. no core structure. Although the claimed variant also claimed that is from

the wild type major coat protein of a virus, i.e. phage display systems, that include the phage display systems of a filamentous phage, a lambda phage, a Baculovirus, a T4 phage and a T7 phage, this limitation is not sufficient to provide a core structure since there is a substantial variation within the genus of these 'different' phage display systems, i.e. a filamentous phage, a lambda phage, a Baculovirus, a T4 phage and a T7 phage, such that the structure of the wild type major coat proteins among these 'different' phage display systems are structurally distinct from each other, i.e. there is no structural nexus among there wild type major coat proteins of these 'different' phage display systems. Accordingly, in the absence of sufficient recitation of distinguishing identifying characteristics, the specification does not provide adequate written description of the claimed genus for the claimed variant.

Thus, the specification does not teach the broad genus for the claimed variant of wild type major coat protein of phage display systems that include the phage display systems of a lambda phage, a Baculovirus, a T4 phage and a T7 phage.

Vas-Cath Inc. v. Mahurkar, 19 USPQ2d 1111, makes clear that "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession *of the invention.* The invention is, for purposes of the 'written description' inquiry, *whatever is now claimed.*" (See page 1117.) The specification does not "clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed." (See page 1116.).

With the exception of the variant of wild type major coat protein of a filamentous phage, i.e. the coat protein VIII of a filamentous phage, disclosed by the specification, the skilled artisan cannot envision the instant claimed fusion protein comprising a heterologous polypeptide and

any variant of any wild type major coat protein of phage display systems that includes the display systems of a lambda phage, a Baculovirus, a T4 phage and a T7 phage. Adequate written description requires more than a mere statement that it is part of the invention and reference to a potential method for making it. See Fiers v. Revel, 25 USPQ2d 1601, 1606 (CAFC 1993) and Amgen Inc. V. Chugai Pharmaceutical Co. Ltd., 18 USPQ2d 1016. In Fiddes v. Baird, 30 USPQ2d 1481, 1483, claims directed to mammalian FGF's were found unpatentable due to lack of written description for the broad class. The specification provided only the bovine sequence.

Finally, University of California v. Eli Lilly and Co., 43 USPQ2d 1398, 1404, 1405 held that:

> ...To fulfill the written description requirement, a patent specification must describe an invention and do so in sufficient detail that one skilled in the art can clearly conclude that "the inventor invented the claimed invention." *Lockwood v. American Airlines, Inc.*, 107 F.3d 1565, 1572, 41 USPQ2d 1961, 1966 (1997); In *re Gosteli*, 872 F.2d 1008, 1012, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989) (" [T]he description must clearly allow persons of ordinary skill in the art to recognize that [the inventor] invented what is claimed."). Thus, an applicant complies with the written description requirement "by describing the invention, with all its claimed limitations, not that which makes it obvious," and by using "such descriptive means as words, structures, figures, diagrams, formulas, etc., that set forth the claimed invention." *Lockwood*, 107 F.3d at 1572, 41 USPQ2d at 1966.

In the present instance, the specification does not teach instant claimed fusion protein comprising a heterologous polypeptide and any variant of any wild type major coat protein of phage display systems that includes the display systems of a filamentous phage, a lambda phage, a Baculovirus, a T4 phage and a T7 phage. Therefore, only the fusion protein comprising a heterologous polypeptide and the variant of wild type major coat protein of a filamentous phage, specifically the coat protein VIII, but not the full breadth of the claimed product meet the written description provision of 35 U.S.C 112, first paragraph.

17. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

18. Claims 3 and 44 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

a) Claim 3 recites the limitation of "the phage" in line 1. There is insufficient antecedent basis for this limitation in claim 1. Claim 1 recites a Markush group regarding the type of variant of a wild type major coat protein of virus, i.e. '*a virus selected from the group consisting of a filamentous phage, a lambda phage, a Baculovirus, a T4 phage and a T7 phage*'.

b) The phrase "the major coat protein" of both claims 3 and 44 vague because it is unclear if it is referring to the '*variant of a wild type major coat protein*' or the '*wild type major coat protein*'. Consequently, the phrase "the major coat protein" of both claims 3 and 44 vague and both claims 3 and 44 are rejected under 35 U.S.C. 112, second paragraph.

c) Claim 4 recites the limitation of "the major coat protein" in line 1. There is insufficient antecedent basis for this limitation in claim 1. Claim 1 recites '*a wild type major coat protein*'.

Claim Rejections - 35 USC § 102

19. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –

> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

20. Claims 1, 8, 9, 11, 12, 30, 46, and 47 are rejected under 35 U.S.C. 102(e) as being anticipated by Larocca et al. (US Patent 6,451,527 B1; *effective filing date of 08/29/199*).

> *The instant invention recites a fusion protein, i.e. a product. Structurally, the product comprises a heterologous polypeptide fused to at least a portion of a variant of a wild type major coat protein of a virus. The variant of a wild type major coat protein of a virus is selected from the group consisting of a filamentous phage, a lambda phage, a Baculovirus, a T4 phage and a T7 phage.*

Larocca et al. disclose a genetic package display system and the method of using it (see e.g. Abstract; col. 2, lines 14-63; col. 3, lines 3-19). The genetic package display system comprises a ligand fused to the phage coat protein (see e.g. col. 3, lines 39-40; col. 4, line 64-65; col. 9, lines 57-65; fig. 1B). The ligand includes foreign protein, peptides, antibodies, or cDNA (refers to instant claimed heterologous polypeptide and instant claims 8, and 46)(see e.g. col. 4, line 64-65; col. 5, line 54 thru col. 6, line 62; col. 9, lines 57-65). In addition the ligand can bind to target such as erbB3 (refers to instant claim 47)(see e.g. col. 11, lines 18-30). The phage coat protein comprises a wild type major coat protein such as filamentous phage, lambda phage, and T4 phage (refers to instant claim 30)(see e.g. col. 4, line 66 thru col. 5, line 53) or mutant coat protein such as the mutant filamentous phage coat protein VIII (refers to instant claimed variant of a wild type major coat protein of a virus and the elected species of filamentous phage of gp VIII)(see e.g. col. 9, lines 36-44). The transformation of the genetic package display system uses host cell such as bacteria host cell (refers to instant claims 9 and 11)(see e.g. col. 7, lines 15-22; col. 10, lines 46-63; col. 11, lines 1-17; col. 17, lines 1-31) and include viral replication system

(refers to instant claim 12)(see e.g. col. 7, line 33 thru col. 8, line 6). Therefore, the genetic package display system of Larocca et al. anticipates the presently claimed fusion protein.

Claim Rejections - 35 USC § 103

21. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

22. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

23. Claims 1, 3, 8, 9, 11, 12, 30-32, 44, 46, and 47 are rejected under 35 U.S.C. 103(a) as being unpatentable over Light, II et al. (US Patent 5,770,356) in view of Larocca et al. (US Patent 6,451,527 B1; *effective filing date of 08/29/199*).

> *The instant invention recites a fusion protein, i.e. a product. Structurally, the product comprises a heterologous polypeptide fused to at least a portion of a variant of a wild type major coat protein of a virus. The variant of a wild type major coat protein of a virus is selected from the group consisting of a filamentous phage, a lambda phage, a Baculovirus, a T4 phage and a T7 phage.*

Light, II et al. disclose a fusion protein (see e.g. Abstract; col. 1, lines 13-17; col. 3, lines 31-38; col. 9, lines 3-5, and 32-35). The fusion protein comprises a heterologous polypeptide attached to the filamentous phage membrane coat protein, and the attachment is through a polypeptide linker at the carboxy terminus of the heterologous polypeptide (see e.g. col. 9, lines 3-5, and 32-35; col. 10, lines 13-34; col. 10, line 58 to col. 11, line 20). The filamentous phage includes phage such as M13, f1, and fd, and membrane coat protein include coat protein such as a gene VIII coat protein (see e.g. col. 10, lines 13-34; col. 13, lines 41-45; col. 22, line 50 thru col. 12). The expression vectors of the host cell encode the fusion protein (see e.g. col. 19, lines 6-41).

The fusion protein of Light, II et al. differs from the presently claimed invention by failing to disclose a variant major coat protein.

Larocca et al. disclose a genetic package display system and the method of using it (see e.g. Abstract; col. 2, lines 14-63; col. 3, lines 3-19). The genetic package display system comprises a ligand fused to the phage coat protein (see e.g. col. 3, lines 39-40; col. 4, line 64-65; col. 9, lines 57-65; fig. 1B). The ligand includes foreign protein, peptides, antibodies, or cDNA (refers to instant claimed heterologous polypeptide and instant claims 8, and 46)(see e.g. col. 4, line 64-65; col. 5, line 54 thru col. 6, line 62; col. 9, lines 57-65). In addition the ligand can bind to target such as erbB3 (refers to instant claim 47)(see e.g. col. 11, lines 18-30). The phage coat protein comprises a wild type major coat protein such as filamentous phage, lambda phage, and T4 phage (refers to instant claims 30 and 31)(see e.g. col. 4, line 66 thru col. 5, line 53) or mutant coat protein such as the mutant filamentous phage coat protein VIII (refers to instant claimed variant of a wild type major coat protein of a virus and the elected species of filamentous phage

of gp VIII)(see e.g. col. 9, lines 36-44). In addition, the mutant coat protein increases transduction efficiency (refers to instant claim 32)(see e.g. col. 9, lines 36-44; especially col. 9, lines 40-44). The transformation of the genetic package display system uses host cell such as bacteria host cell (refers to instant claims 9 and 11)(see e.g. col. 7, lines 15-22; col. 10, lines 46-63; col. 11, lines 1-17; col. 17, lines 1-31) and include viral replication system (refers to instant claim 12)(see e.g. col. 7, line 33 thru col. 8, line 6).

It would have been obvious to a person of ordinary skill in the art at the time the invention was made to disclose a variant major coat protein as taught by Larocca et al. in the fusion protein of Light, II et al. One of ordinary skill in the art would have been motivated to disclose a variant major coat protein in the fusion protein of Light, II et al. for the advantage of providing a coat protein that allows for more rapid uncoating and increased transduction capacity (Larocca: col. 9, lines 40-44) since both Light, II et al. and Larocca et al. disclose filamentous phage wild type major coat protein such as M13, gene III, and gene VIII (Light, II: col. 10, lines 13-34; Larocca: col. 4, line 66 thru col. 5, line 1). Furthermore, one of ordinary skill in the art would have a reasonable expectation of success in the combination of Light, II et al. and Larocca et al. because the type of major coat protein use in the fusion protein would be a choice of experimental design and is considered within the purview of the cited prior art.

Therefore, the combine teachings of Light, II et al. and Larocca et al. do render the fusion protein of the instant claims *prima facie* obvious.

24. Claims 1, 7-9, 11, 12, 30-32, 46, 47, 55, and 58 are rejected under 35 U.S.C. 103(a) as being unpatentable over Larocca et al. (US Patent 6,451,527 B1; *effective filing date of 08/29/199*) in view of Li et al. (*J. Biol. Chem.*, **1993**, 268(7), pgs. 4584-4587).

> *The instant invention recites a fusion protein, i.e. a product. Structurally, the product comprises a heterologous polypeptide fused to at least a portion of a variant of a wild type major coat protein of a virus. The variant of a wild type major coat protein of a virus is selected from the group consisting of a filamentous phage, a lambda phage, a Baculovirus, a T4 phage and a T7 phage.*

Larocca et al. disclose a genetic package display system and the method of using it (see e.g. Abstract; col. 2, lines 14-63; col. 3, lines 3-19). The genetic package display system comprises a ligand fused to the phage coat protein (see e.g. col. 3, lines 39-40; col. 4, line 64-65; col. 9, lines 57-65; fig. 1B). The ligand includes foreign protein, peptides, antibodies, or cDNA (refers to instant claimed heterologous polypeptide and instant claims 8, and 46)(see e.g. col. 4, line 64-65; col. 5, line 54 thru col. 6, line 62; col. 9, lines 57-65). In addition the ligand can bind to target such as erbB3 (refers to instant claim 47)(see e.g. col. 11, lines 18-30). The phage coat protein comprises a wild type major coat protein such as filamentous phage, lambda phage, and T4 phage (refers to instant claims 30 and 31)(see e.g. col. 4, line 66 thru col. 5, line 53) or mutant coat protein such as the mutant filamentous phage coat protein VIII (refers to instant claimed variant of a wild type major coat protein of a virus and the elected species of filamentous phage of gp VIII)(see e.g. col. 9, lines 36-44). In addition, the mutant coat protein increases transduction efficiency (refers to instant claim 32)(see e.g. col. 9, lines 36-44; especially col. 9, lines 40-44). The transformation of the genetic package display system uses host cell such as bacteria host cell (refers to instant claims 9 and 11)(see e.g. col. 7, lines 15-22; col. 10, lines 46-

63; col. 11, lines 1-17; col. 17, lines 1-31) and include viral replication system (refers to instant claim 12)(see e.g. col. 7, line 33 thru col. 8, line 6).

The fusion protein of Larocca et al. differs from the presently claimed invention by failing to disclose the amino acid substitution for the variant of a wild type major coat protein.

Li et al. disclose mutant M13 coat protein (see e.g. Abstract; pg. 4584, right col., lines 6-37; pg. 4585, fig. 1). The mutagenesis comprises randomized oligonucleotides annealed to either the wild type major coat protein of M13 or the mutant of the wild type major coat protein of M13 and the transformation step use *E coli* as the host cell (see e.g. pg. 4584, right col., lines 40-69; pg. 4585, left col., lines 8-19). The amino acid substitution ranges from 2 to 13 and the site of the mutation is at residues numbers 22, 23, 24, 27, 28, 29, 30, 31, 32, 33, 36, 37, and 38 (see e.g. pg. 4584, right col., line 40 thru pg. 4585, left col., line 6; pg. 4585, left col., lines 19-47; pg. 4585, fig. 1).

It would have been obvious to a person of ordinary skill in the art at the time the invention was made to disclose the amino acid substitution for the variant of a wild type major coat protein as taught by Li et al. in the fusion protein of Larocca et al. One of ordinary skill in the art would have been motivated to disclose the amino acid substitution for the variant of a wild type major coat protein in the fusion protein of Larocca et al. for the advantage of providing a major coat protein with alter species distributions and protein-protein interaction within the transmembrane region (Li: pg. 4586, right col., lines 5-11) since both Larocca et al. and Li et al. disclose mutant filamentous phage major coat protein VIII (Larocca: col. 9, lines 40-44; Li: pg. 4585, fig. 1). Furthermore, one of ordinary skill in the art would have a reasonable expectation of success in the combination of Larocca et al. and Li et al. because Li et al. disclose the success

of the amino acid substitution for the variant of a wild type major coat protein for use in mutagenesis (Li: pg. 4585, right col., lines 3-14).

Therefore, the combine teachings of Larocca et al. and Li et al. do render the fusion protein of the instant claims *prima facie* obvious.

Withdrawn Objection(s) and /or Rejection(s)

25. The rejection of claims 1, 3, 4, 7, 9, 11, 12, 30, 31, and 44 under 35 USC 102(b) as being anticipated by Light, II et al. (US Patent 5,770,356) has been withdrawn in light of applicant's amendments of claim 1.

26. The rejection of claims 1, 3, 4, 7-9, 11, 12, 30-33, and 44-47 under 35 USC 103(a) as being obvious over Light, II et al. (US Patent 5,770,356) and Marks et al. (US Patent 6,794,128 B2) has been withdrawn in view of applicant's amendments of claim 1.

Response to Arguments

27. Applicant's arguments with respect to claims 1, 3, 4, 7-9, 11, 12, 30-33, and 44-47 have been considered but are moot in view of the new ground(s) of rejection.

Conclusion

28. No Claims allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to My-Chau T. Tran whose telephone number is 571-272-0810. The examiner can normally be reached on Monday: 8:00-2:30; Tuesday-Thursday: 7:30-5:00; Friday: 8:00-3:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Andrew J. Wang can be reached on 571-272-0811. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

My-Chau T. Tran
February 27, 2006

Date Mailed: November 18, 2005

Sheet 1 of 1

FORM PTO-1449 INFORMATION DISCLOSURE STATEMENT IN AN APPLICATION (Use several sheets if necessary)	Docket Number: 11669.141USWO	Application Number: 09/380,447
	Applicant: SIDHU ET AL.	
	Filing Date: 09/01/1999	Group Art Unit: 1639

U.S. PATENT DOCUMENTS

EXAMINER INITIAL	DOCUMENT NO.	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
MC	5,571,689	11/05/1996	HEUCKEROTH ET AL.			
	5,627,024	05/06/1997	MARUYAMA ET AL.			
	5,663,143	09/02/1997	LEY ET AL.			
MC	5,766,905	06/16/1998	STUDIER ET AL.			
	~~6,054,312~~	~~04/25/2000~~	~~LAROCCA ET AL.~~	Duplicate of		PTO-1449
	~~6,190,908 B1~~	~~02/20/2001~~	~~KANG~~	filed 2/26/04		

FOREIGN PATENT DOCUMENTS

	DOCUMENT NO.	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION	
						YES	NO
MC	WO 95/34683	12/21/1995	PCT				

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)

MC		Efimov, V. et al., "Bacteriophage T4 as a Surface Display Vector", *Virus Genes,* 10:173 (1995)
		Ren, Z. et al., "Phage T4 SOC and HOC display of biologically active, full-length proteins on the viral capsid", *Gene,* 215:439-444 (1998)
		Ren, Z. et al., "Phage display of intact domains at high copy number: A system based on SOC, the small outer capsid protein of bacteriophage T4", *Protein Science,* 5:1833-1843 (1996)
MC		Smith, G. et al., "Libraries of Peptides and Proteins Displayed on Filamentous Phage", *Methods in Enzymology,* 217:228-257 (1993)

EXAMINER	DATE CONSIDERED 2/23/06

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; draw line through citation if not in conformance and not considered. Include copy of this form for next communication to the Applicant.

Date Mailed: FEBRUARY 26, 2004

Sheet 1 of 1

INFORMATION DISCLOSURE STATEMENT IN AN APPLICATION (Use several sheets if necessary)	Docket Number: 11669.141USWO	Application Number: 09/380,447
	Applicant: SIDHU ET AL.	
	Filing Date: 09/01/1999	Group Art Unit: 1639

U.S. PATENT DOCUMENTS

EXAMINER INITIAL	DOCUMENT NO.	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
MAR	6,054,312	04/25/2000	Larocca et al.			
MAR	6,190,908 B1	02/20/2001	Kang			

FOREIGN PATENT DOCUMENTS

	DOCUMENT NO.	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION YES	TRANSLATION NO

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)

MAR		Cason, J. et al., "Identification of Immunogenic Regions of the Major Coat Protein of Human Papillomavirus Type 16 that Contain Type-restricted Epitopes," *Journal of General Virology*, Vol. 70, Part 11, pp. 2973-2987 (November 1989)
		Hasan, N. et al., "Boundaries of the nutL antiterminator of coliphage lambda and effects of mutations in the spacer region between boxA and boxB," *Gene*, Vol. 50, Nos. 1-3, pp. 87-96 (1986)
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		Vogel, M. et al, "Production of a recombinant antigen of Echinococcus multilocularis with high immunodiagnostic sensitivity and specificity," *Molecular and Biochemical Parasitology*, Vol. 31, pp. 117-125 (1988)
MAR		Walker, S. et al., "ToxR (RegA)-mediated in vitro transcription of *Pseudomonas aeruginosa toxA*," *Gene*, Vol. 150, pp. 87-92 (1994)

23552 PATENT TRADEMARK OFFICE		DATE CONSIDERED 2/23/06

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; draw line through citation if not in conformance and not considered. Include copy of this form for next communication to the Applicant.

*Substitute Disclosure Statement Form (PTO-1449)

Notice of References Cited

Application/Control No.	Applicant(s)/Patent Under Reexamination	
09/380,447	SIDHU ET AL.	
Examiner	Art Unit	Page 1 of 1
MY-CHAU T. TRAN	1639	

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-5,770,356 A	06-1998	Light et al.	435/5
*	B	US-6,451,527 B1	09-2002	Larocca et al.	435/6
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Li et al., "Conformational states of mutant M13 coat proteins are regulated by transmembrane residues", 3/5/1993, J. Biol. Chem., 268(7), pgs. 4584-4587.
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Communication

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 268, No. 7, Issue of March 5, pp. 4584–4587, 1993

Conformational States of Mutant M13 Coat Proteins Are Regulated by Transmembrane Residues*

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Mutational and structural analysis of the 28 viable bacteriophage M13 mutants obtained by randomized mutagenesis of the effective transmembrane (TM) segment of the 50-residue major coat (gene VIII) protein (residues 21–39) demonstrated that M13 coat protein functionality, as reflected by phage viability, is incompatible with an increase in Gly + β-branched residue content in its TM core. SDS-polyacrylamide gel electrophoresis and circular dichroism spectroscopy performed in membrane environments on purified mutant coat proteins revealed that these proteins exist in a range of state(s), identified as helical monomers and dimers and polymeric (α-helical and/or β-sheet) species, of which relative populations, and thermally induced conformational transitions, were dependent uniquely upon mutation type and locus. Mutations to relatively polar residues (*e.g.* G23D, Y24D, Y24H, A27E, I32T, and T36S) stabilized principally monomeric species, while mutants with decreased β-branched content in the protein TM hydrophobic core (*e.g.* V29A, V30A, V31A, V31L, and V33A) displayed mainly dimeric species. Mutation of Ile^{37} → Thr within a "Sternberg-Gullick" consensus sequence of the coat protein TM segment led to a highly oligomerized/polymerized protein. The overall results suggest that TM residues in M13 coat protein are not universal components of a hydrophobic anchor segment *per se*, but are further selected (i) to impart conformational flexibility to the TM segment through helix destabilization and (ii) to retain the capacity to regulate protein-protein association and packing motifs within membranes.

Transmembrane (TM)[1] domains in integral membrane proteins are helical segments often regarded essentially as hydrophobic membrane anchors, yet their primary sequences (1) contain up to 40–50% residues considered in soluble proteins to be α-helix breakers (Gly) and β-sheet promoters (Val, Ile, Thr) (2). Since interactions *in vivo* modulated by TM regions in single-spanning membrane proteins are frequently central to function (*e.g.* translocation (Ref. 3); receptor signal transduction (Refs. 4 and 5); ion transport (Ref. 6); virus transformation *via* a cytokine receptor (Ref. 7)), membrane-buried Gly and β-branched residues may have a regulatory role in such conformation-dependent biological events (8).

The requirement for transitions among conformational states is epitomized by the 50-residue major (gene VIII) coat protein during the life cycle of bacteriophage M13, a single-stranded DNA phage with its genome enveloped by a capsid consisting of ~2700 copies of this protein (with limited copies of minor coat proteins) (3, 9, 10). DNA-bound M13 coat proteins are essentially 100% α-helical (11), and helical aggregates of coat proteins are believed to be intermediate states in the host inner membrane during phage assembly (12, 13). However, the β-sheet (*i.e.* "extended") conformation is proposed to be favored for membrane proteins while they are in aqueous compartments prior to membrane insertion (14). The concentration- and temperature-dependent transitions from α-helices to β-sheet structures observed for WT coat (12, 15) provide additional evidence for the conformational flexibility of the mature WT sequence. Wild type (WT) coat protein has been widely studied in membrane-mimetic environments as a model single-spanning membrane protein (9, 11, 15–19). As 12 of the 19 residues in the effective TM segment of M13 coat protein (residues 21–39: YIGYAWAMVVVIVGATIGI) (9) consist of Gly + β-branched residues, we sought to determine the limits within which phage viability might be compromised by manipulation of TM Gly, Ile, Val, and Thr residue content. We now report that point mutations in M13 coat TM segment regulate (and perturb) a balance among conformational states that may underlie functional protein-protein interactions.

MATERIALS AND METHODS

Mutagenesis was performed using an oligonucleotide-directed mutagenesis kit, based on the Eckstein method (20), obtained from Amersham Corp. M13 mp18 single-stranded DNA (originally obtained from Pharmacia, Uppsala, Sweden) was typically used as mutagenesis template. Synthesized oligonucleotides were purified by either PAGE-urea electrophoresis or by oligonucleotide purification cartridges (Applied Biosystems, Foster City, CA). Generally, *Escherichia coli* JM-101 strain was used as host cell in the transformation step. To assess general toleration of M13 phage to TM mutations, "randomized" oligonucleotides were used, which contained 92.5% wild type precursor at each selected site but also 2.5% "contaminants" of each of the other three nucleotides (21). Three such randomized oligonucleotides were prepared, which in combination encompassed the full M13 coat TM region, each 22 nucleotides long with overlaps of 4 nucleotides. Oligonucleotide sequences were: 5′-CGCCCACGC-ATAACCGATATAT-3′ (oligonucleotide 1; coat protein residues 21–27); 5′-GACAATGACAACAACCATCGCC-3′ (oligonucleotide 2; residues 27–33); and 5′-GATACCGATAGTTGCGCCGACA-3′ (oligonucleotide 3; residues 33–39); italicized bases were not randomized. Following mutagenesis experiments, a total of 575 plaques resulting from several transformations were sequenced. The total was comprised of 140 plaques derived from transformation of oligonucleotide 1 mutagenesis products, of which 66 (including silent) mutants were identified (efficiency, 47.9%); 285 plaques derived from oligonucleotide 2, of which 53 mutants were identified (18.6%); and 150 plaques derived from oligonucleotide 3, from which 13 mutations were identified (8.7%). Eight double (including silent) mutants were harvested with mutations 1–5 nucleotides apart. The overall mutagenesis efficiency was 23%. No preference for any specific nucleotide changes was observed. Several positions, including Gly^{23}, Tyr^{24}, Ala^{25}, Ala^{27}, Val^{29}, Val^{30}, and Val^{31}, proved relatively susceptible to nucleotide

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‡ To whom correspondence should be addressed.

[1] The abbreviations used are: TM, transmembrane; WT, wild type; PAGE, polyacrylamide gel electrophoresis.

changes (10–20 mutations were identified at each of these positions, including several isolates of the same mutant), yet among these, Ala^{25} was highly conserved to actual amino acid substitutions. Each mutant phage was amplified, and each of the 28 corresponding coat proteins was isolated and purified in milligram quantities for gel and spectroscopic analysis.

RESULTS AND DISCUSSION

Through site-directed mutagenesis using randomized oligonucleotides annealed to an M13 mp18 single-stranded DNA template (22) (see also Ref. 23), 28 viable mutant M13 phages were generated (Fig. 1), 24 with single-site and four with double-site mutations, within the putative TM region of their respective coat proteins. Although all mutant phages have plaque-forming ability, and all 28 mutant coat proteins could be isolated and purified in milligram amounts, preliminary results from assays of overall phage amplification efficiency ("relative viability") based on limited contact with host (24) indicated that the majority of the 28 phages were deficient (most between 5 and 50%) *versus* WT (100%). The overall scope of substitutions indicates that viable mutants accept significant variations in coat protein hydrophobicity, side chain chemistry, and molecular volume, although tolerance to hydrophilic substitutions is low in a hydrophobic core segment (residues 28–39). A preponderance of polar substitutions at the Gly^{23},Tyr^{24} locus (Fig. 1) has suggested (22) that this diad is periplasm-exposed and that the coat protein TM segment actually enters the host membrane with its first helical turn near Ala^{25}-Trp^{26}-Ala^{27}-Met^{28}. This observation is consistent with amide proton exchange rate analysis of WT coat reported by Henry and Sykes (19) and parallels the finding in soluble proteins that frequent, often polar, viable mutations correlate with residue surface accessibility (25). β-Branched and Gly content in the TM hydrophobic core must already be near-maximal in WT phage, as no mutants were obtained in this region with increased Gly or increased β-branched residue content. Several positions (Ala^{25}, Trp^{26}, Gly^{34}, and Ala^{35}) were found to be conserved to any kind of single-site mutations, and Gly^{38} could accommodate only Ser, consistent with the fact that these residues tend to be conserved throughout the filamentous phage class I family (26). Evidence that this 28-mutant library is representative of the full range of mutations compatible with phage viability is the fact that a wide number of point mutations having equal statistical weight to those observed, which involve a single nucleotide change (*e.g.* Ala^{27} → Gly, Val, or Pro; Val^{29}, Val^{30}, or Val^{31} → Gly or Phe), did not arise, while many of the observed mutations arose repeatedly. Viable TM mutations of M13 coat protein are thus permissive, but not random.

WT coat, deduced to exist as a helical dimer in 2.5 mM SDS solutions (9, 19), migrates at 25 °C as a diffuse dimer/monomer mixture under the SDS-PAGE gel conditions shown in Fig. 2. Yet most mutants differed from WT in this experiment; several migrated on gels predominantly as monomers (G23D, G23S-A25S, Y24D, Y24H, A27E, I32T, and T36S), while

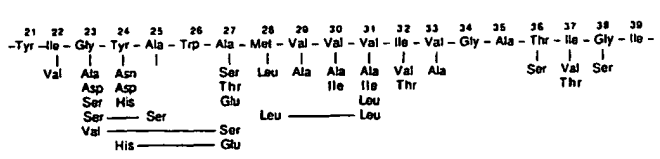

FIG. 1. **Viable transmembrane segment mutants obtained for bacteriophage M13 coat protein.** See also Ref. 22. Each amino acid entered *below* the WT sequence represents a single mutant at the indicated position. Double-site mutants are indicated by *connecting lines*. The full M13 coat protein sequence is AEGDDPAKAAFNSLQASATEYIGYAWAMVVVIVGATIGIKLFKKFTSKAS (9).

several others behaved as dimers (M28L-V31L, V29A, V30A, V31A, V31L, and V33A) (compare Y24D and V31L gels in Fig. 2). When we examined the relative stabilities of these species for the full library on gels after incubation of proteins for 1 and 4 h at 65 °C, WT M13 coat protein and monomer-forming mutants displayed deposits of polymeric β-sheet aggregates; in contrast, dimer-forming mutants exhibited no trace of β-sheet formation at 65 °C (typified by Y24D and V31L, respectively, in Fig. 2). Gel patterns (not shown) further revealed that several mutants (*e.g.* I22V, G23A, G23S, G23V-A27S, Y24N, A27S, I37T (see below), I37V, and G38S) displayed oligomeric bands in the range of hexameric (30 kDa) and octameric (40 kDa) molecular mass in addition to major monomer and/or dimer species. Such oligomeric species, which likely correspond to the "b state" of WT coat, are stable helical aggregates of dimers (15). Populations of oligomeric species on gels were not affected by extended heating at 65 °C.

Circular dichroism (CD) (27) spectra of membrane-solubilized coat protein samples corresponding to those used in SDS-PAGE gel experiments confirmed the α-helical structures for monomeric (Y24D) and dimeric (V31L) species at 25 °C, and the course of the transitions to β-structures for WT and Y24D at 65 °C (Fig. 2). In related experiments, we determined $\alpha \rightarrow \beta$ transition temperatures for the full mutant library by monitoring the temperature dependence of the CD helical parameter $[\theta]_{208}$. All 28 mutants retained the inherent capacity to exist in multiple (α, β) conformational states; however, mutations had profound effects on transition temperature values, which ranged between 62 and 95 °C. WT coat was centrally located at 75.6 °C; monomer-forming mutants typically underwent the transition at ~70 °C, whereas dimer-forming mutants typically displayed the transition at ~90 °C. This wide range of $\alpha \rightarrow \beta$ transition temperatures may be attributable to the fact that extant populations of dimers first "melt" to monomers, which are the species that undergo thermally induced onset of β-sheet aggregation. In contrast to these findings for membrane-bound coats, transition temperatures of DNA-bound proteins were mutation-independent, namely a group of intact phages (see also Ref. 28) representative of the full range of transition temperatures of membrane-bound coats (studied by CD as 1 mg/ml solutions in Tris-HCl/EDTA buffer, pH 8) had identical $\alpha \rightarrow \beta$ transition temperatures (88.7 ± 0.2 °C). Given that all 28 mutants retain the basic C-terminal segment involved in the DNA-coat protein interface (10, 23), this common transition point for DNA-bound proteins indicates that viability necessitates formation of a unique conformation, ostensibly 100% α-helix (11), for DNA-bound coat.

Primary sequence within the M13 coat protein transmembrane segment thus regulates the relative populations in the membrane environment of helical monomeric, dimeric, and oligomeric species, and their capacities to inter-equilibrate. TM point mutations exert their greatest effect on inter-chain structural and packing interactions (analogously to mutations in the two-stranded α-helical coiled-coil (Ref. 29); see also glycophorin (Ref. 30)). Generally, mutations to more polar residues produced coat protein monomers, presumably because they promote a more aqueous average environment for such mutants (8); mutations involving reduction in β-branched content (notably Val → Leu or Ala) led to predominantly dimeric species, suggesting a discrete role for β-branching in destabilization of helix-helix aggregation. Oligomeric species, which can be seen, *e.g.*, in mutant I37T as a striated pattern of highly associated species (Fig. 3), may represent an *in vitro* visualization of intermediates(s) in the

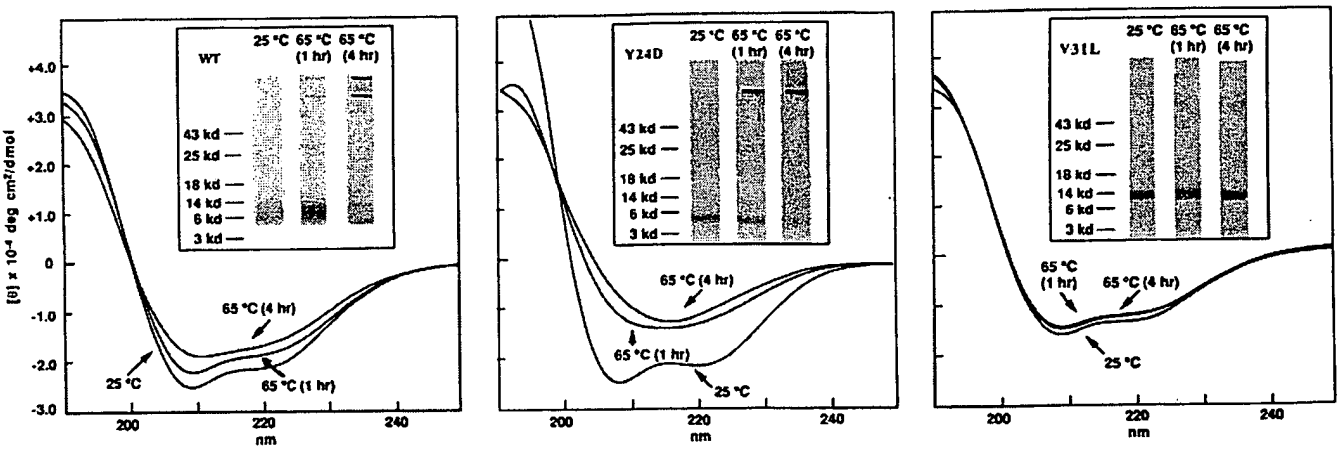

FIG. 2. **Relative thermal stabilities of α-helical species of wild type M13 coat protein and selected transmembrane segment mutants, determined by SDS-PAGE gel electrophoresis and circular dichroism spectroscopy.** Panels show (from *left* to *right*) WT coat protein; Y24D, a typical monomer-forming mutant; and V31L, a typical dimer-forming mutant (see text). The molecular mass of coat protein monomer is 5.2 kDa. Protein (4 μg) was loaded in each sample well in a total volume < 10 μl. Gels were run in buffer containing 0.025 M Tris, 0.192 M glycine, and 0.1% SDS at pH 8.3. CD spectra (4–6 scans for each protein) were obtained on a Jasco J-720 instrument using a quartz cell with path length of 0.1 mm. CD measurements were performed on coat protein samples (1 mg/ml) in 30 mM sodium deoxycholate containing 25 mM sodium borate, pH = 9. Coat protein samples were incubated at 65 °C at the indicated time periods before recording a CD spectrum or loading on the gel. β-Aggregation is seen for WT both on top of the gel and at the interface between the 5% stacking gel and the 15% separating gel.

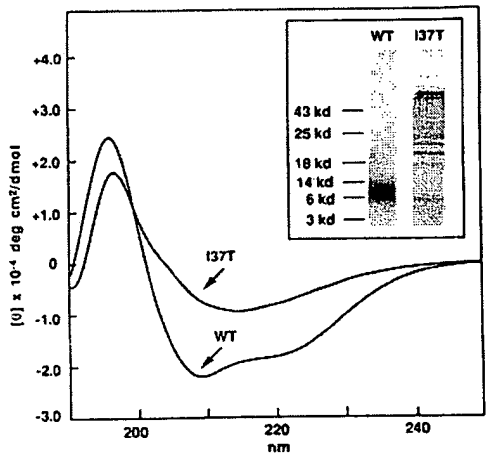

FIG. 3. **SDS-PAGE gels and circular dichroism spectra at 25 °C of wild type and M13 coat protein mutant I37T.** Experimental details are as given in the legend to Fig. 2.

host inner membrane which mediate DNA extrusion during *in vivo* phage assembly (13). We noted in addition that the M13 coat protein TM segment contains a "Sternberg and Gullick" (31) consensus sequence (Gly^{34}-Ala-Thr-Ile-Gly^{38}) of the type suggested to regulate protein-protein association in the EGF receptor family, namely mutation of $Val^{664} \rightarrow$ Glu within such a consensus sequence (Ala^{661}-Thr-Val-Val-Gly^{665}) in *neu* gene results in a constitutively activated oncogenic receptor (32). The combination of α-oligomeric + β-sheet species and largely β-sheet CD spectrum at 25 °C observed for mutant I37T (Fig. 3) similarly supports a role for this sequence in nucleating intramembranous helix-helix association. Such poorly reversible protein-protein association may result from introduction of the Thr^{37} OH group, which could promote strong H-bonding to a carbonyl oxygen in a neighboring protein chain. Mutation-resistant M13 coat residues Gly^{34} and Gly^{38} are located on a common face in a schematic helical wheel (22), further implicating a key role for these small residues in protein-protein packing motifs.

Notwithstanding a general feature of membrane proteins that many residues in TM helices act as "low information" (*e.g.* Ref. 33) hydrophobic anchor sites, we find that viability imposes limits for residue selection within the M13 TM sequence, particularly with respect to location and maximal content of Gly and β-branched residues. That single-point TM mutations can alter species distributions and protein-protein interactions within the TM region suggests that the membrane-spanning segment of M13 coat protein, and likely those of membrane proteins possessing a single TM span, can be considered as functional domains that preserve the capacity for structural interconversions. This situation contrasts with α-helices as structural components of soluble proteins, where primary sequences may already be optimized for helical stability (34).

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